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UPTAKE OF LEUCINE AND WATER BY *CENTRUROIDES SCULPTURATUS* (EWING) EMBRYOS (SCORPIONES, BUTHIDAE)

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ABSTRACT

In vitro radiotracer studies using tritiated leucine revealed that *C. sculpturatus* embryos accumulated leucine by a process that is characterized by a rate constant of approximately 0.02 min^{-1}. Cyanide inhibits leucine uptake, indicating that active transport is probably involved. Leucine is incorporated into embryonic proteins, and uptake is more rapid during early developmental stages when growth is most rapid. Although leucine uptake continues throughout development, dry mass of embryos does not increase during the later stages. These stages are characterized by differentiation, maturation, and by accumulation of water. Total body water increases from 53% of body mass in embryos with unpigmented median eyes to more than 80% at birth.

INTRODUCTION

The data on embryology and parturition in scorpions was recently reviewed by Francke (1982), who concluded that the term ovoviviparous could not justifiably be applied to scorpions (see also Williams 1969). Instead, he argued that the terms katoikogenic and apoikogenic, first suggested by Laurie (1896), were more appropriate. Katoikogenic scorpions (Diplocentridae and Scorpionidae) are characterized by relatively small eggs, lack of persistent extraembryonic membranes, long parturition times, and nutrition of the embryos via a placenta-like interaction between the uterine wall and the oral cavity of the embryos. Apoikogenic scorpions (Buthidae, Chactidae, Iuridae, and Vaejovidae) are characterized by well-developed extraembryonic membranes (the amnion and serosa) that fuse to enclose completely the embryo, short parturition times, and relatively large eggs. It has generally been assumed that apoikogenic scorpion embryos receive no nourishment from the mother, and thus the term ovoviviparous has been applied, in spite of the fact that embryonic growth in apoikogenic scorpions results in considerable increase in size (Laurie 1896).

Preliminary work in my laboratory had revealed that tritium-labelled leucine (^{3}H-leucine) injected into the hemolymph of gravid female *Centruroides sculpturatus* could be detected in the embryos within 72 hours. The experiments described in this paper were undertaken to analyze the kinetics of leucine entry into the embryos and to determine whether the leucine is actually incorporated

into the embryonic proteins. Data on mass changes during development were also gathered.

MATERIALS AND METHODS

Gravid female *C. sculpturatus* were collected from a population occupying cobblestone beaches along the Salt River approximately 50 km east of Phoenix, Arizona and returned immediately to the laboratory. All scorpions were maintained at 25°C and given *ad libitum* access to food (crickets) and drinking water.

Uptake of Radiolabeled Leucine (^{3}H-leucine).—Embryos were obtained from females that had been sacrificed by severing their anterior mesosoma. Embryos were dissected from the ovariuterus and placed in scorpion ringers (pH 7.4, 25°C) solution (Ahearn and Hadley 1976). Only embryos with intact extraembryonic membranes and, with one exception (see "^{3}H-leucine Uptake by Early Embryos," below), pigmented median eyes were used.

Kinetics of ^{3}H-leucine Uptake.—The time-course of ^{3}H-leucine penetration into the embryos was determined by placing three embryos into each of seven tubes containing 2.0 ml aliquants of scorpion ringers to which had been added ^{3}H-leucine and ^{131}I-albumin (ICN Radiochemicals). At intervals (see Table 1), the embryos in a tube were removed and their extraembryonic membranes were dissected free and discarded. The embryos were briefly washed with a gentle stream of distilled water and then homogenized in a known volume (1.88-2.20 ml) of distilled water. The ^{3}H and ^{131}I activity in a 1.0 ml aliquant of each homogenate was determined by liquid-scintillation counting. The total ^{3}H activity for each set of 3 embryos was then calculated by correcting the observed counting rate for the total volume of the respective homogenates.

Two replicate experiments were performed, the first one day, and the second two days after collection.

Effect of Metabolic Inhibition of Embryos.—To determine if the uptake of ^{3}H-leucine was energy-dependent, during each replicate experiment an additional tube containing 2.0 ml of radiolabeled scorpion ringers and sufficient NaCN (aqueous solution) to yield a CN^- concentration of 1 mM was set up. Three embryos were placed in the tube and after 120 min incubation (at 25°C) the embryos were homogenized and ^{3}H activity was determined as described above.

Incorporation of ^{3}H-leucine into Embryonic Proteins.—As part of the second replicate experiment, a tube containing 2.0 ml of ^{3}H-leucine ringers and three embryos was incubated (at 25°C) for 48 h. The embryos were then homogenized in 0.5 ml distilled water and the proteins in a 40 μl aliquant of the homogenate were precipitated with 10% trichloroacetic acid (TCA). The precipitated proteins were washed with 5% TCA and ethanol/ether (50/50, v/v). The washed proteins were solubilized and ^{3}H activity was determined. Total ^{3}H activity in the proteins of the three embryos was then estimated as described above.

^{3}H-leucine Uptake by Early Embryos.—One of the females sacrificed for the first replicate contained embryos that were at a very early developmental stage as evidenced by their small size and the lack of visible differentiation of a metasoma or appendages. Three of these embryos (with intact extraembryonic

membranes) were placed into 2.0 ml of ^{3}H-leucine ringers and uptake was determined after 60 min incubation.

Mass Changes During Embryogenesis.—Seven of the gravid females were sacrificed by exposure to CN^-, and their embryos were removed and dissected free of their extraembryonic membranes. Each female's clutch of embryos was assigned to one of two developmental classes, based on whether or not the median eyes were pigmented (metasomal and appendage development was evident in all embryos). The total mass of each clutch was immediately determined to the nearest 0.1 mg, after which the embryos were freeze-dried to a constant mass.

Five females were allowed to deliver broods. After all of the young had climbed to their mother's dorsum, the total wet and dry masses of the newborn scorpions and their mothers were determined as described above.

Statistical comparisons involving the data were accomplished with appropriate non-parametric and parametric tests.

RESULTS

^{131}I counting rates did not significantly exceed background counting rates in any set of embryos, indicating that the extraembryonic membranes were intact and that large molecules were unable to penetrate the membranes at significant rates.

The time-course of entry of ^{3}H-leucine into *C. sculpturatus* embryos is indicated in Table 1. In both replicates, embryo counting rates (CPM) increased rapidly during the first 5 minutes, with a declining rate of increase thereafter. The presence of the metabolic inhibitor cyanide in the incubation medium significantly reduced the rate of leucine entry; even after 120 min incubation, embryo CPM were less than 1/3 those of the normal embryo 45 min CPM ($t = 5.20$, $p \ll 0.001$). Leucine entry into the early embryos was more rapid, as evidenced by the more than two-fold higher CPM in the earlier embryos.

After 48 h incubation in medium containing ^{3}H-leucine, the embryonic proteins are definitely labelled with ^{3}H, indicating that the ^{3}H-leucine is incorporated into the embryonic proteins.

Estimates of rate constants for entry of leucine into the embryos may be obtained from the data in Table 1. The time-dependent increase in concentration of a solute entering a reservoir is described by the equation:

$$\ln \frac{c_\infty - c_t}{c_\infty} = -kt \qquad \text{Equation 1}$$

where c_∞ and c_t are, respectively, the solute concentrations in the reservoir at time $= \infty$ (i.e., at equilibrium) and at time t after initiation of the experiment, and k is the rate constant (Kotyk and Janacek 1975). Equation 1 also describes the time-dependent increase in counting rate (CPM) when radiotracers are used to monitor transport processes. In the present context, k is a measure of how rapidly the amino acid concentration in the embryos approaches equilibrium with the incubation medium. Numerically, it equals the proportion by which the difference between c_t and c_∞ is reduced each minute. Because c_t approached c_∞

Table 1.—Uptake of ^{3}H-leucine by *C. sculpturatus* embryoes. CPM = ^{3}H counts per minute for 3 embryos.

	First Replicate		Second Replicate	
Incubation Time (min)	CPM	% of Medium CPM[1]	CPM	% of Medium CPM[2]
2.5	273	0.32	380	0.53
5.0	408	0.48	702	0.97
10.0	701	0.83	618	0.86
20.0	942	1.12	842	1.17
30.0	848	1.01	997	1.38
45.0	1182	1.40	1182	1.64
60.0	1348	1.60	—[3]	—
CN^- (120 min)	369	0.44	375	0.52
Protein Incorporation (48 h)	—	—	13,238	18.34
Early Embryos (60 min)	2972	3.53	—	—

[1]Medium CPM = 42,125 CPM ml^{-1}
[2]Medium CPM = 36,084 CPM ml^{-1}
[3]Sample destroyed during processing

asymptotically, I used an iterative approach to estimate the value of c_∞ that maximized the r^2-value when Equation 1 was fitted to the data in Table 1 by least-squares regression. The CPM values for t = 2.5 min were not used in the regression analysis, because the data suggested the presence of a rapidly exchanging compartment that resulted in anomalously high CPM at t = 2.5 min. The results of this analysis are presented in Table 2.

Similarly, estimates of k for CN^--poisoned embryos may be obtained from Equation 1. Here, however, the estimates are biased somewhat by the fact that the effects of the rapidly-exchanging compartment cannot be factored out, and the values of k in Table 2 are probably somewhat larger than they should be.

In a strict sense, the fact that the leucine is incorporated into proteins requires use of a more complicated model (Kotyk and Janacek 1975). The consequence of not including the incorporation process in the model is that the estimated values of k for 'uninhibited' embryos (Table 2) are somewhat too high. The r^2-values suggest, however, that the errors are not large enough to preclude use of the k-values for purposes of discussion.

Wet and dry masses of different ontogenetic stages are presented in Table 3. Dry mass did not change significantly during the developmental stages listed, but

Table 2.—Kinetic analysis of ^{3}H-leucine uptake by *C. sculpturatus* embryos. Parameters were derived by iterative fitting of the data in Table 1 using Equation 1 as a model for least-squares regression.

	C_∞ (CPM)	k(min^{-1}	r^2	k(CN^-)
First Replicate	1660	0.023	0.94	.0021
Second Replicate	1700	0.018	0.95	.0021

Table 3.—Ontogenetic changes in wet and dry mass of individual *C. sculpturatus* embryos. Values are presented as $\bar{x} \pm$ S.D.. Total number of offspring in the litters is given in parentheses below the number of litters. Litter sizes ranged from 19 to 34 ($\bar{x}$ = 26.6, S.D. = 5.30).

Development stage	Number of litters	Average Dry Mass (mg)	Average Wet Mass (mg)	% H_2O
Median eyes unpigmented	3 (78)	2.70 ± 0.46	5.77 ± 1.04	53.1 ± 0.6
Pigmented median eyes	4 (121)	2.65 ± 0.64	9.99 ± 2.29	73.3 ± 1.5
Newborn	6 (147)	2.50 ± 0.15	12.72 ± 1.45	80.8 ± 0.7
Adults	—	—	—	71.2 ± 1.2

wet masses increased significantly (Kruskal-Wallis single factor ANOVA; H =9.45, $p < 0.01$). This reflected a greater than 3-fold increase in the water content of embryos, from an average of 3.07 mg H_2O in embryos with unpigmented median eyes to 10.22 mg H_2O at birth.

DISCUSSION

Leucine not only penetrates the extraembryonic membranes of *C. sculpturatus* embryos, but is also incorporated into the proteins of the embryos at a fairly rapid rate (Table 1). Presumably, other amino acids and nutrients such as lipids and carbohydrates are also accumulated and utilized by the embryos. In the case of leucine, at least, the accumulation is energy-dependent, as evidenced by the order of magnitude decrease in k, the rate constant for leucine uptake, brought about by the addition of cyanide to the incubation medium (Table 2). These findings suggest that *C. sculpturatus* embryos are not metabolically isolated from their mother, but rather, are adapted to utilize nutrients provided by her throughout embryonic development. From that perspective, *C. sculpturatus* (and probably other apoikogenic scorpions) is not qualitatively different from the katoikogenic scorpions, in spite of the lack of obvious morphological specializations for embryo nutrition.

Ontogenetic changes in amino acid uptake are also suggested by the data in Table 1. It is not possible to calculate a rate constant for leucine transport in the "Early Embryo" stage, but the 60 min CPM is significantly greater than the corresponding CPM for the embryos whose median eyes have become pigmented (i.e., the stage used for all other uptake measurements listed in Table 1). This indicates that leucine uptake during the early stages of embryonic development is more rapid than during later stages. As is discussed below, the early developmental stages of *C. sculpturatus* are characterized by considerable increase in dry mass, while the later stages grow very little, if at all. The higher leucine uptake rate in the "Early Embryos" undoubtedly reflects this.

The nature of the energy-dependent process responsible for leucine uptake is unclear. The energy-dependence could result from active transport across the extraembryonic membranes, active transport into the embryonic tissues,

incorporation of leucine into the embryonic proteins, or a combination of these. The data presented here do not allow discrimination among the possibilities. In mammalian small intestine, transmembrane flux of alanine (like leucine, a neutral, lipophilic amino acid) results from a combination of passive diffusion along concentration gradients and active transport. At low luminal alanine concentrations, active transport is essential for amino acid uptake, whereas at higher concentrations, passive diffusion becomes the predominant uptake mode (Stevens, Kaunitz and Wright 1984).

The presence of ^{3}H-leucine in the embryos that had been incubated with cyanide indicates that leucine can diffuse through the extraembryonic membranes and into the embryonic tissues (Tables 1 and 2). However, the large effect of cyanide on the value of k (Table 2) makes it seem unlikely that the energy dependence of leucine uptake stems solely from the incorporation of leucine into proteins. Active transport through the extraembryonic membranes is probably involved, although further work is necessary to test this hypothesis.

Accumulation of dry mass is essentially completed by the time most of the characteristic features of scorpion external morphology (metasoma, pedipalps, etc.) have developed (Table 3). Growth during stages is considerable. If we assume that *C. sculpturatus* oocytes are spherical and similar in dimension to those reported for *C. vittatus* (approximately 0.5 mm; Francke 1982), and have a specific gravity of 1.0, then an oocyte would have a total (wet) mass of approximately 65 μg. By the earliest developmental stage listed in Table 3, even dry mass has increased by at least 40-fold. Dry mass changes very little during the later stages, indicating that differentiation and maturation, rather than growth, predominate.

There is, however, a marked increase in water content of embryonic *C. sculpturatus* during the final phase of development. Water content of the average embryo increases by more than 7 mg prior to birth. This results in an increase in the % H_2O from 53.1% to more than 80% at birth. Comparable data for earlier developmental stages of other arthropods do not appear to be available, so the generality of the increase in water content cannot be assessed. Water absorption, apparently energy-dependent, does occur in insect eggs, however (Edney 1977). The mechanism of H_2O accumulation in embryonic *C. sculpturatus* is unknown, but the constancy of dry mass of the embryos suggests an extraembryonic source.

The water content of newborn *C. sculpturatus* is higher than that of most other arthropods, being comparable to levels reported for lepidopteran larvae (Edney 1977). The increase in water content prior to birth may be adaptive. Newborn scorpions are, in many respects, extrauterine embryos: the sting is enclosed in a 'sheath', the cuticle is not sclerotized or tanned, and the young apparently do not feed while on the mother's back. The cuticle of newborn scorpions is probably relatively ineffective at retarding water loss. Prior to leaving the mother and beginning independent existence, the young molt. Molting scorpions seem to be very susceptible to relative humidity (O. F. Francke, personal communication), and successful completion of molting may be impossible if the scorpion desiccates too much prior to molting. The relatively high water 'stores' of newborn scorpions may thus increase the probability of completing the first molt.

The water stores could also be critical to the survival of the first instar larvae after leaving the mother. Organisms as small as first instar *C. sculpturatus* have

high surface:volume ratios, which makes the danger of fatal desiccation particularly acute. Because of their flattened shape, scorpions have relatively large surface areas for a given mass (Toolson and Hadley 1977), which would tend to exacerbate the susceptibility of the first instar young to transcuticular water losses. I suspect that desiccation is a leading cause of mortality for immature scorpions, particularly during the first instar. Although I do not have evidence that the high water content of the newborn scorpions is maintained through the molting to first instar, if it were this would certainly enhance the probability of surviving.

The data presented in this paper are consistent with Francke's contention that the term ovoviviparous should not be applied to the Buthidae or other apoikogenic families of scorpions. Although the details of the uptake processes remain to be worked out, it is apparent that the embryos of *C. sculpturatus,* at least, are not nutritionally isolated from their mother. Embryos accumulate leucine at the expense of energy, particularly during the earlier developmental stages (when most of the increase in embryonic mass occurs), and incorporate the leucine into proteins. Even during the later developmental stages, leucine uptake continues but at reduced rates. Moreover, during the final developmental stages, the embryos accumulate large amounts of water, which must necessarily be derived, at least in part, from the water stores of the mother.

The demonstration that 'large-egged' apoikogenic scorpions provide nutrients to the embryos raises some interesting questions about the evolution of viviparity in the order. Little work has been done on the metabolism of gravid females or of embryos. In the katoikogenic scorpion, *Heterometrus fulvipes* (C. L. Koch), a member of the family Scorpionidae, gravid females exhibit enhanced glycolytic activity in the hepatopancreas and "reproductive tissues" (Jayaram et al. 1978). In the same tissues, total lipid content and total protein content decrease significantly during embryogenesis apparently to meet the metabolic demands of the embryos. Comparable work on apoikogenic scorpions does not appear to have been done. Comparative studies on several species should be undertaken. The resulting data should provide insights into the reason(s) why the two markedly different 'approaches' to embryogenesis have been maintained during the evolution of the Scorpiones.

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